

**Supplementary Information for:**

**Probing the evolutionary history of human bitter taste receptor pseudogenes by restoring their function**

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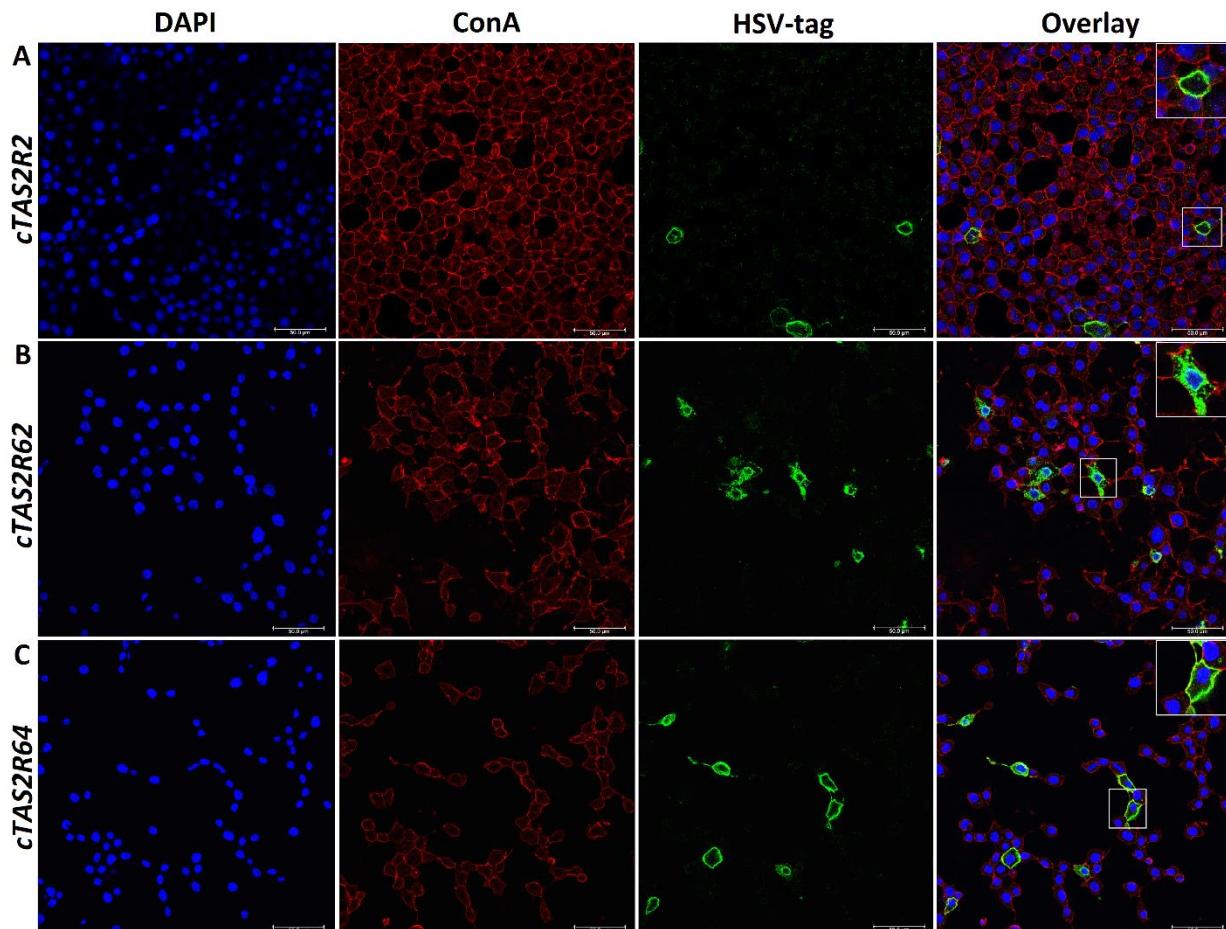
**Supplementary Table S1**

**Supplementary Table S2**

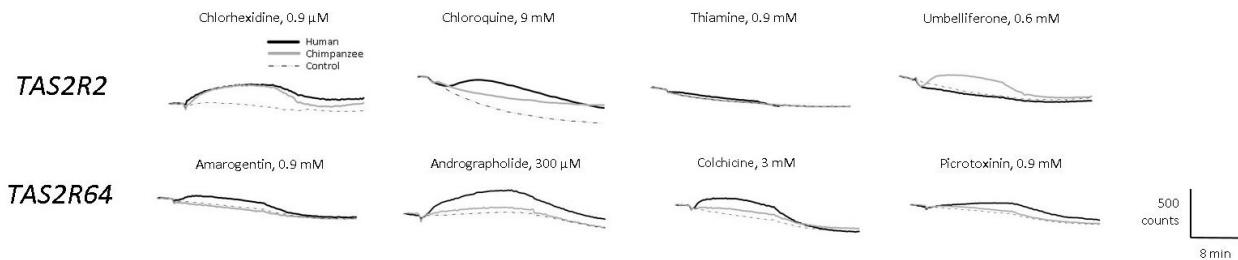
**Supplementary Table S3**

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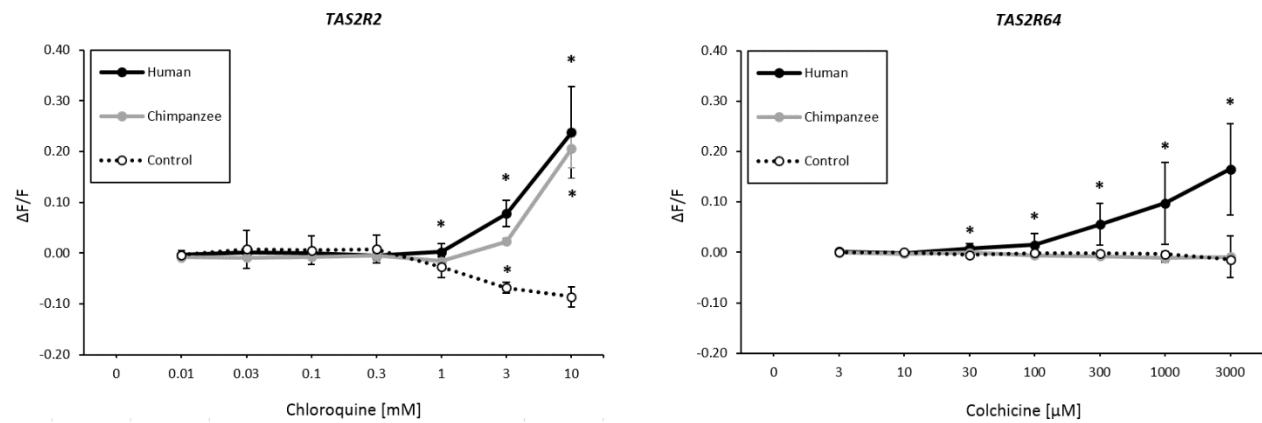
**Supplementary Figure S1.** Confocal fluorescence images of HEK293T cells transfected with chimpanzee *TAS2R2*, *TAS2R62* and *TAS2R64* receptors and negative control transfected cells. Receptor expression is visualized by a mouse anti-HSV glycoprotein D antibody combined with an Alexa488- labeled anti-mouse antibody (green). Cell surface is visualized by plasma membrane glycoproteins detected with biotin-conjugated concanavalin A and avidin-conjugated Texas Red (red). Cell nuclei are visualized with 4',6-diamidino-2-phenylindole (DAPI) (blue).



**Supplementary Figure S2.** Identification of agonists for human (black solid line) and chimpanzee (grey solid line) *TAS2R2* and *TAS2R64*. Responses of the negative control transfected cells are shown with dotted lines.



**Supplementary Figure S3.** Dose-response relationships of human (black circles) and chimpanzee (grey circles) *TAS2R2* and *TAS2R64* receptors with increasing concentrations of chloroquine and colchicine, respectively. Responses of negative controls transfected cells are shown with white circles. The y axis shows the relative fluorescence changes ( $\Delta F/F$ ), and the x axis is labeled with the relative chloroquine and colchicine concentrations. Asterisks indicate a significance difference from control.



**Supplementary Figure S4.** Amino acid sequences of the synthetized human (h) and chimpanzee (c) *TAS2Rs*.

>*hTAS2R2*

MALSFSAILHIIIMMSAEFFTGITVNGFLIIVNCNELIKHRKLMPIQILLCIGMSRGQLQMVLVQSFFSVFFPLLYVKIIYG  
AAMMFLWMFFSSISLWFATCLSVYCLKISGFTQSCFLWLKFRIPKLIPWLLGSVLASVIASVCIEVDYAKNVEEDALR  
NTTLKKSHTKIKKISEVLLVNLALIFPLAIFVMCTSMLLISLYKHTHRMQHGSHGRNANTEAHINALKTITFFCFISYFAA  
FMTNMTFSLPYRSHQFFMLKDIMAAYPSGHHSVIIILNSNSKFQQSFRRLCLKKKL

>*cTAS2R2*

MALSFSAVLHIIIMMSAEFFTGITVNGFLIIVNCNELIKHRKLMPVQILLCIGMSRGQLQMVLVQTFFSVFFPLLYVKIIY  
GAAMMFLWMFFSSISLWFATCLSVYCLKISGFTQSCFLWLKFRIPKLIPWLLGSVLASVIASVCIEVDFAKNVEEDAL  
RNNTLKKRKAKIKKISEVLLVNLALIFPLAIFVMCTSMLLISLYKHTHRMQHGSHGRNANTEAHINALKTITFFCFISYF  
AAFMTNMTFSLPYRSHQFFMLKDIMAAYPSGHHSVIIILNSNSKFQQSFRRLCLKKKL

>*hTAS2R62*

MPSLPTLIFIAIFCLES LAAM LQNGFLVTMLGREWVRCMLSTSDMIVACLAASRFCLHGVAMVNLLASLDFSRAVPY  
MNIFWDLFNALT LWFTALLA AFYCVKISSFSHPTFAWLKRWRNSRLVPKLIKGS LIICGLEV ISSATGNILFGQRKVSLSSYR  
NETLVYRVQASFQLYFFLYEGFVWSILFLFLVSTVLLIVSLCWQLGQMRDRLRPGPCDPSTQAYTMALKSLTFSLIFCTLYF  
LSLFASALKIINFQNHW HWAWEVLIYANICLHSTV LVRSPKLKGLKTWPQLQCPRAAGSQGFGRCWP

>*cTAS2R62*

MPSLPTLIFIAIFCLES LAAM LQNGFLVTMLGREWVRCMLSTSDMIVACLAASRFCLHGVAMANNLLASLDFSRAVPY  
MNIFWDLFNALT LWYTALLA AFYCVKISSFSHPTFAWLKRWRNSRLVPKLIKGS LIICGLEV ISSATGNILFGQRKVSLSSYR  
ETLVYRVQASFQLYFFLYDG FVWSIPFLFLVSTVLLIVSLCWQLGQMRDRLRPGPCDPSTQAYTMALKSLTFSLIFCTLYF  
SLFASALKIINFQNHW HWAWEVLIYANICLHSTV LVRSPKLKGLKTWPQLQCPDAGSQGFGRCWP

>*hTAS2R64*

MVYFLLIILSILVVFAFVLGNFSNGFIALVNVIDWVKTRKISSADQILTALVVS RIGLLWVILLHWYANVFNSALYSSEVGAV  
ASNISAIINHSIWLAASLSIFYLLKIANFSNLIFHLKKRIRSVVLVILLGPLVFLICNLAVITMDDSVWTKEYEGNVTKIKL  
RNAIHLNSLT VST LANLIP FILT LICFL LLICSLH KHLKKMQLHGKG SQDLST KVHI KAL QTV ISFLML YAIYFLYLT LTWNL  
TQQNKLVFL CQ TLGIMYPSFH SFFL IMGSRKLKQTFLS VLCQV T CLVK GQQP STP

>*cTAS2R64*

MVYFLLIILSILVVFAFVLGNFSNGFIALVNVIDWVNTRKISSADQILTALVVS RIGLLWVILFHWYANVFNSALYSSEVGAV  
VASNISAIINHSIWLAASLSIFYLLKIANFSNLIFHLKKRIRSVVLVILLGPLVFLICNLAVITMDERWTKEYEGNVTKIKL  
LRNAIHLSDLTVST LANLIP FILT LICFL LLICSLH KHLKKMQLHGKG SQDLST KVHI KAL QTV ISFLML YAIYFLYLT LTWNL  
WTQQNKLVFL CQ TLGIMYPSFH SFFL IMGSRKLKQTFLS VLCQV T CLVK GQQP STP

**Supplementary Table S1.** Codon position and receptor location of the human-chimpanzee amino acid changes in *TAS2R2*, *TAS2R62* and *TAS2R64* genes.

TM, trans-membrane domain; ec, extra-cellular loop; ic, intra-cellular loop.

Gene	Position	Change	Location
<i>TAS2R2</i>	8	I/V	TM1
	13	M/I	TM1
	45	I/V	TM2
	155	Y/F	ec2
	172	S/R	ec2
	174	T/A	ec2
<i>TAS2R62</i>	64	V/A	TM2
	121	N/I	TM4
	181	E/D	TM5
	188	L/P	TM5
	300	R/C	C terminus
	301	A/D	C terminus
<i>TAS2R64</i>	37	K/N	ic1
	64	L/F	TM2
	151	D/E	TM4
	152	S/R	ec2
	176	N/D	TM5

**Supplementary Table S2.** Average transfection rates (expressing cells/total cells) of both human (h) and chimpanzee (c) *TAS2R2*, *TAS2R62* and *TAS2R64* genes.

Construct	Average (%) Transfection Rate
<i>hTAS2R2</i>	10.2 +/- 5.2
<i>cTAS2R2</i>	3.6 +/- 0.6
<i>hTAS2R62</i>	11.0 +/- 0.7
<i>cTAS2R62</i>	9.2 +/- 1.2
<i>hTAS2R64</i>	15.1 +/- 4.9
<i>cTAS2R64</i>	11.4 +/- 0.9
Mock	0

**Supplementary Table S3.** PCR and sequencing primers used to amplify and sequence *hTAS2R2*, *hTAS2R62* and *hTAS2R64* pseudogenes.

Primer name	Direction	Sequence	Reaction
<i>hTAS2R2_END</i>	Reverse	TGCTGCCATTATGTCCTTCA	PCR, Sequencing
<i>hTAS2R2_END</i>	Forward	TGGCTTAGAAATGCCAACAA	Sequencing
<i>hTAS2R2_MID</i>	Forward	GTTCTGGCCTCTGTGAGCAT	Sequencing
<i>hTAS2R2_MID</i>	Reverse	TGGGATCCTGAATTCAACC	Sequencing
<i>hTAS2R2_START</i>	Forward	ATGCCCTTGCTTTTCAGC	PCR, Sequencing
<i>hTAS2R2_START</i>	Reverse	AAAGGAACATCATTGCTGCAC	Sequencing
<i>hTAS2R62_END</i>	Reverse	CTATGCCAGCACCTTCC	PCR, Sequencing
<i>hTAS2R62_END</i>	Forward	TTCAGAACATCACTGGCACTGG	Sequencing
<i>hTAS2R62_MID</i>	Forward	GCAGGCTTCATTCAGCTCT	Sequencing
<i>hTAS2R62_MID</i>	Reverse	TTCCCAGTGGCTGATGAGAT	Sequencing
<i>hTAS2R62_START</i>	Forward	TGCCCACGTTGATCTTCATA	PCR, Sequencing
<i>hTAS2R62_START</i>	Reverse	GCATTGAAAAGGTCCCAGAA	Sequencing
<i>hTAS2R64_END</i>	Reverse	CCTGCTTCCCATAATCAGGA	PCR, Sequencing
<i>hTAS2R64_END</i>	Forward	TCAGCACCAAGGTCCACATA	Sequencing
<i>hTAS2R64_MID</i>	Forward	TGGGTCCCTGGTATTTTG	Sequencing
<i>hTAS2R64_MID</i>	Reverse	GGGTCAGAACATGAAGGGTATGA	Sequencing
<i>hTAS2R64_START</i>	Forward	GGAAATTTTCCAATGGCTTC	PCR, Sequencing
<i>hTAS2R64_START</i>	Reverse	AGCAAGCCAGATGCTGAAAT	Sequencing

**Supplementary Table S4.** List of the 83 natural and synthetic bitter compounds used to challenge human and chimpanzee *TAS2Rs*.

Compound	Tested concentrations for initial screening
1,10-Phenanthroline	3 mM, 0.9 mM
6-propyl-2-thiouracil (PROP)	3 mM, 0.9 mM
Aristolochic acid	30 µM, 9 µM
Absinthin	900 µM, 300 µM
Acesulfame K	30 mM, 9 mM
Acetaminophen	9 mM, 3 mM
Acetylpyrazine	3 mM, 0.9 mM
Allylisothiocyanate	900 µM, 300 µM
Aloin	30 µM, 9 µM
Amarogentin	3 mM, 0.9 mM
Andrographolid	900 µM, 300 µM
Androsterone	3 µM, 0.9 µM
Arbutin	90 mM, 30 mM
Atropine	30 mM, 9 mM
Azathioprine	900 µM, 300 µM
Bacitracine	90 µM, 30 µM
Brucine	300 µM, 90 µM
Caffeine	900 µM, 300 µM
Camphor	3 mM, 0.9 mM
Carisoprodol	300 µM, 90 µM
Chenodesoxycholic acid	3 µM, 0.9 µM
Chininsulfat	30 µM, 9 µM
Chloramphenicol	3 mM, 0.9 mM
Chlorhexidine	3 µM, 0.9 µM
Chloroquine	30 mM, 9 mM
Chlorpheniramine	300 µM, 90 µM
Colchicine	9 mM, 3 mM
Cortisone	9 µM, 3 µM
Coumarin	900 µM, 300 µM
Creatinine	9 mM, 3 mM
Cromolyn	30 mM, 9 mM
Cucurbitacin I	300 µM, 90 µM
Curcumin	300 µM, 90 µM
Cyclamate	90 mM, 30 mM
Cycloheximid	3 mM, 0.9 mM
Denatonium benzoate	9 mM, 3 mM
Digitonin	3 µM, 0.9 µM
Dimethyl sulfoxide (DMSO)	3%, 0.9%
Diphenhydramine	30 µM, 9 µM
Diphenidol	300 µM, 90 µM
Emetine	90 µM, 30 µM

Enterolactone	30 $\mu$ M, 9 $\mu$ M
Epicatechin	3 mM, 0.9 mM
Epigallocatechin gallate	30 $\mu$ M, 3 $\mu$ M
Erythromycin	900 $\mu$ M, 300 $\mu$ M
Falcarindiol	300 $\mu$ M, 90 $\mu$ M
Famotidine	900 $\mu$ M, 300 $\mu$ M
Genistein	30 $\mu$ M, 9 $\mu$ M
Ginkgolide A	900 $\mu$ M, 300 $\mu$ M
Limonin	900 $\mu$ M, 300 $\mu$ M
L-Phenylalanine	3 mM, 0.9 mM
L-Tryptophan	3 mM, 0.9 mM
Naringin	30 $\mu$ M, 9 $\mu$ M
Nicotine	300 $\mu$ M, 90 $\mu$ M
Noscapine	30 $\mu$ M, 9 $\mu$ M
Ouabain	9 mM, 3 mM
Pantothenic acid	30 $\mu$ M, 9 $\mu$ M
Papaverin	90 $\mu$ M, 30 $\mu$ M
Parthenolide	300 $\mu$ M, 90 $\mu$ M
Phenylthiocarbamide (PTC)	300 $\mu$ M, 90 $\mu$ M
Picrotin	3 mM, 0.9 mM
Picrotoxinin	3 mM, 0.9 mM
Pirenzepine	30 mM, 9 mM
Progesterone	9 $\mu$ M, 3 $\mu$ M
Quassassin	900 $\mu$ M, 300 $\mu$ M
Resveratrol	30 $\mu$ M, 9 $\mu$ M
Riboflavin	30 nM, 9 nM
Saccharin	30 mM, 9 mM
Salicin	30 mM, 9 mM
Sinigrin	3 mM, 0.9 mM
Sodium benzoate	6 mM, 2 mM
Sparteine	900 $\mu$ M, 300 $\mu$ M
Strychnine	90 $\mu$ M, 30 $\mu$ M
Sucrose octaacetate (SOA)	900 $\mu$ M, 300 $\mu$ M
Taurine	300 mM, 90 mM
Taurocholic acid	900 $\mu$ M, 300 $\mu$ M
Theobromine	3 mM, 0.9 mM
Thiamin	3 mM, 0.9 mM
Umbelliferone	1.8 mM, 0.6 mM
Xanthotoxin	300 $\mu$ M, 90 $\mu$ M
Yohimbine	900 $\mu$ M, 300 $\mu$ M
$\alpha$ -Solanine	3 $\mu$ M, 0.9 $\mu$ M
$\alpha$ -Thujone	3 mM, 0.9 mM